RESEARCH
IN
PLANT TRANSPIRATION: 1963

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RESEARCH IN PLANT TRANSPIRATION: 1963

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INTRODUCTION

The transfer of water from the soil to the atmosphere by evaporation from leaf surfaces accounts for a large part of the loss of water from land. The quantity of water delivered to the atmosphere by evaporation from leaves is large and much of it may be an unnecessary loss when viewed from the standpoint of plant production. This report presents results from a portion of the continuing effort by the Agricultural Research Service research group at the Southern Piedmont Conservation Research Center to discern factors affecting transpiration and to develop methods for control of transpirational loss of water by plants.

A report by USAEPG (*45*)[1] of research conducted in 1960 described the principal characteristics and capabilities of a controlled environment growth room with high light intensity where many of the studies reported here were conducted.

The report of 1961 studies in USDA Production Research Report No. 70 (*26*) presented prediction equations to account for approximately 80 percent of the observed variations in transpiration rates. This report also emphasized the dependence of transpiration rate on soil moisture availability and the extraordinary ability of guard cells to remain operative under adverse conditions. The pathway of guard cell starch accumulation was partially elucidated, and results with several compounds having potential as antitranspirants were recorded.

Further studies in 1962 were reported in USDA Production Research Report No. 87 (*23*). The 1962 report includes a description of light distribution in the growth room and studies of transpiration rates using grain sorghum and corn with various combinations of light, temperature, humidity, and soil moisture tension. Also reported was further experimentation with foliarly applied chemicals for control of transpiration by plants.

Results of studies conducted in 1963 are reported herein. This report includes a discussion of the characteristics and capabilities of small portable growth chambers where several of the experiments were conducted.

Results of studies with bean and tomato plants under two light sources illustrate the effects of light quality on growth and development of plants.

This report calls attention to the effects of CO_2 concentration of the air and soil moisture tension on transpiration, leaf temperature, and stomatal activity of several agronomic crops.

Some insight into the relation of protoplasmic streaming and guard cell action is provided. Also included are negative results obtained with soil-applied Atrazine[2] and soil and foliar applications of hexadecanol-octadecanol for decreasing transpiration and increasing water use efficiency by plants.

CONTROLLED ENVIRONMENT STUDIES

Growth Chambers

Discrepancies found in the literature frequently can be traced to lack of detailed reporting. This research report contains detailed descriptions of facilities and techniques because the authors believe such treatment is necessary to avoid misunderstanding.

An experiment has real value only if it can be reproduced. This is not always possible, even with the best equipment available today. Much remains to be done in the science of artificial climate control.

The first annual report of research in plant transpiration at the Southern Piedmont Conservation Research Center mentioned the use of small environment chambers (*45*, p. 17) as preconditioning units for plant growth. Their use has also been mentioned in subsequent reports (*26*, *23*). It is the purpose of this section to discuss their capabilities and give information for assessment of their limitations.

All growth chambers are limited to some degree in the uniformity of environmental conditions that

[1] Italic numbers in parentheses refer to Literature cited, p. 24.

[2] Trade names and company names are used in this publication solely to provide specific information. Mention of a trade name does not constitute a guarantee or warranty, and does not signify that the product is approved to the exclusion of comparable products.

can be maintained. Precision of control mechanisms is usually the limiting factor. The basic unit of our growth chambers is a U.S. Army walk-in refrigerator (fig. 1) from which the top has been removed and replaced with 1- to 4-mil polyethylene or 5-mil Mylar sheeting. Polyethylene allows CO_2 passage, but its transmission of visible light decreases with age. Mylar does not age appreciably but allows no CO_2 passage to make up deficiencies caused by photosynthesis in the chamber. Inside dimensions are 68 by 72 by 64½ inches and outside dimensions, 76 by 79 by 78 inches.

The compressor unit for cooling is mounted on the rear wall. The original gasoline engine has been replaced by a 1.5–hp. electric motor that drives the compressor on demand and a 0.5–hp. motor that drives the 20-inch-diameter squirrel cage fan located behind the condenser cooling coils (fig. 2). This fan runs continuously, insuring constant air circulation in the chamber. Four-foot-wide tables with adjustable legs have been placed in the middle of the chamber. The typical wind pattern 1 foot above the table and 2 feet from the lamp is shown in figure 3. The data were obtained with a Hastings air meter, Model B15A, with a directional probe, type N–7. Areas with high velocity (>300 ft./min.) are not used for experimental plants.

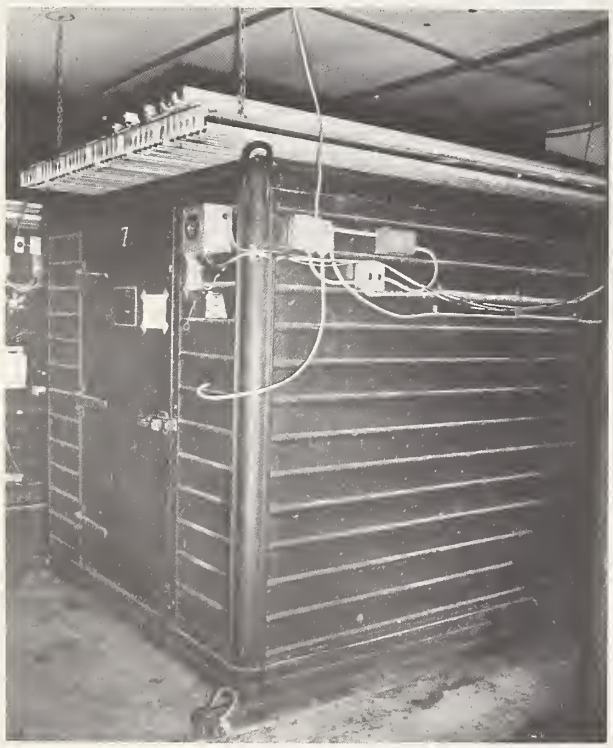

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FIGURE 1.—Front and side view of a growth chamber including overhead light bank. Bank projects over chamber, excluding the use of the burned and blackened fluorescent tube ends.

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FIGURE 2.—Inside view of growth chamber. Condenser cooling coil on rear wall with wind deflector above. Humidifier on shelf in left corner; dehumidifier on floor under table. Cooled air, which is expelled at top of condenser unit, flows into cooling coils below bench height. Edges of cone-type electric heater coils are barely visible above air deflector.

The chambers are capable of maintaining temperatures ranging from 10° to 40° C. ±2° constantly or with diurnal cycling. Humidity is controllable during the photoperiod within the limit of 30 to 90 percent ±10 percent. This is accomplished with a Walton Model SW2 [3] humidifier wired into a Honeywell H63A humidity controller. Dehumidification is obtained by use of self-contained portable dehumidifiers capable of extracting 14 quarts in 24 hours at 80° F., 60 percent relative humidity. A thermostatically activated safety switch interrupts all power to the chamber and triggers an alarm system if internal temperatures exceed or go below the preset limits.

Light is supplied from a bank of cool white VHO fluorescent lamps supplemented with 23 incandescents, if necessary. Incandescents used normally vary from 15 to 75 watts, depending on the species' needs. The fluorescent lights are adequate for

[3] Walton, Inc., Irvington, N.J.

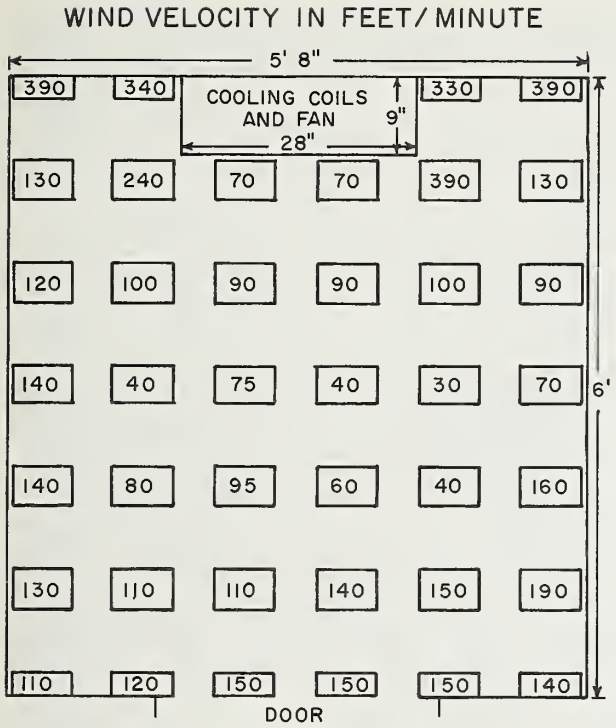

FIGURE 3.—Wind distribution of selected points at 1-foot intervals, 1 foot above bench height in growth chambers. All values are in feet per minute.

normal plant growth of some species without the supplemental incandescents. Figure 4 shows the light distribution of this system at 100 centimeters below the bank or 16 cm. above bench height. Polyethylene sheeting was used in the foregoing light determinations; with the use of Mylar sheeting total radiant energy decreased, whereas visible light increased. The data were gathered from lamps with approximately 500 hours' service. Light output from fluorescent lamps decreases considerably with age; therefore, to help offset this, bulbs are replaced after logging 1,500 hours.

Excellent growth of the species listed below has been attained in the chambers when soil is adequately fertilized and photoperiods, temperature, and humidities are optimum for individual species' needs.

The following are species that were grown successfully in growth chambers made from U.S. Army walk-in refrigerators:

Dicotyledons	*Monocotyledons*
Gossypium hirsutum (cotton)	Zea mays (corn)
Rumex patientia (dock)	Sorghum vulgare (sorghum)
Phaseolus vulgaris (field bean)	Cynodon dactylon (bermudagrass)
Vicia faba (horsebean)	Poa pratensis (Kentucky bluegrass)
Vinca major (periwinkle)	Poa trivalis (Roughstalk bluegrass)
Rheum rhaponticum (rhubarb)	Festuca elatior (tall fescue)
Glycine max (soybean)[4]	Lolium multiflorum (annual ryegrass)
Lycopersicon esculentum (tomato)	Zebrina pendula (wandering-Jew)
Brassica rapa (turnip)	

Comparative Bean and Tomato Growth and Fruiting Under Two Fluorescent Lamp Sources

With the present trend toward controlled environment research, the biologist is frequently confronted with the problem of having to choose a light source for the culture of his plants. Unfor-

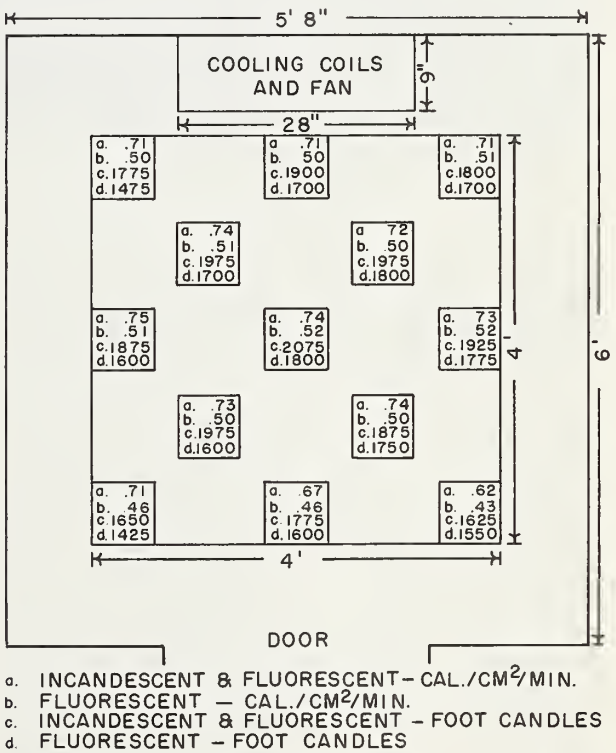

FIGURE 4.—Light distribution at selected points 16 centimeters above bench height or 1 meter from the nearest source.

[4] Will not grow successfully without adding supplemental incandescent lights to the fluorescents.

tunately, little data are presently recorded in the literature to help him reach his decision. Measuring growth rates of plants under a series of light intensities for each light source (*17*) serves a very useful purpose of evaluating growth; however, the effect of lights on flowering and fruiting should also be considered. In some instances physiological abnormalities traceable to light quality may be most easily detected in flowering and fruit set of any one species.

For several years in our research on plant transpiration we have used light banks containing cool white VHO fluorescent lamps (F96T12/CW/VHO)[5] over environmental chambers for the successful culture of a number of plants (see list on p. 3 of this report). With some species, such as soybean, incandescent lighting to supplement the cool white VHO's has been found to be absolutely necessary for optimum growth; other plants, such as field bean and tomato plants, have not required supplementary lighting.

Gro-Lux lamps such as the F20T12/GRO have been widely acclaimed as superior light sources for growth of "shade" plants (gloxinia, African violet, etc.). Lamps with similar emissive characteristics are also available in high-output form, e.g., F96T/GRO/VHO. The question arose whether F96T/GRO/VHO lamps are a better light source than the VHO cool white presently employed for the culture of field crops that normally grow under light of high intensity. A comparison was made therefore between the growth, flowering, and fruiting of bean and tomato plants grown under VHO cool white and VHO Gro-Lux.

The study was conducted in a single growth chamber using two test species: *Lycopersicon esculentum* var. Rutgers, and *Phaseolus vulgaris* var. Red Kidney. Plants were grown in asphalted metal containers with 178 pounds of Cecil sandy loam limed to pH 6.5 and fertilized with 4,000 pounds of 6–12–12 (120 p.p.m. nitrogen (N), 100 p.p.m. phosphorus (P), and 200 p.p.m. potassium (K)) per acre. This procedure insured a uniform aboveground environment and minimized edaphic influences. The test population consisted of four plants of each species under each light source. The soil moisture was monitored throughout the studies by the neutron probe method (*42*) and was maintained above 50 percent available to minimize any moisture tension effect on growth, flowering, or fruiting. The chamber was divided by a large polyethylene sheet hung between the two light sources (fig. 5), which effectively intercepted the light between the two sections but not air movement.

New ballasts and lamps were used. The light source above each section consisted of either sixteen 8-foot VHO cool white lamps or sixteen 8-foot VHO Gro-Lux lamps. No supplemental incandescent light was used. The photoperiod was 14 hours with 25° C. day and 20° C. night temperatures. The net radiant energy from the two light sources was equal at the beginning of the experiment, being 0.68 cal. $cm.^{-2}$ $min.^{-1}$ as measured 6 inches above the soil surface with a Beckman-Whitley Net Radiometer (model N 188–01).

Under both light sources the initial date of flowering for each species was the same—29 days after planting for beans and 47 days after planting for tomatoes.

Table 1 summarizes the data obtained. The differences in growth or fruiting of bean plants, although appearing to favor VHO cool white, were not significantly different at the 5-percent level under the two light sources. The growth, flowering, and fruiting of tomato plants were definitely inferior under F96T/GRO/VHO lamps to those under VHO cool white; all differences were highly significant.

TABLE 1.—*Average yield of red kidney bean plants and Rutgers tomato plants grown under light sources indicated*

Plant	Yield per plant	
	F96T/GRO/VHO	F96T12 CW/VHO
Red kidney beans: [1]		
Fruit—No.	8	11
Fruit, fresh weight—g.	26	32
Plant, fresh weight—g.	65	67
Plant, dry weight—g.	10	12
Rutgers tomatoes: [2]		
Flower—No.	7	12
Fruit—No.	0.25	3
Fruit, fresh weight—g.	13	37
Plant, fresh weight—g.	495	850
Plant, dry weight—g.	46	92

[1] 55 days old.
[2] 82 days old.

Plants grown under F96T/GRO/VHO showed excessive internode elongation when compared with field-grown plants or plants grown under VHO cool white, indicating an improper spectral balance for the culture of those test species.

Unfortunately, man has not yet produced an artificial light source comparable to the sun in intensity and quality. Therefore, considerably more of his attention could be directed toward the necessary research and development of such light sources. The effort put forth in developing lamps

[5] Sylvania Lighting Products Division, Salem, Mass.

FIGURE 5.—Part of test plants as seen inside growth chamber; plants grown under F96T12/CW/VHO on left of plastic barrier (see text for description), plants grown under F96T/GRO/VHO on right. Excessive internode elongation can be noted on both bean and tomato plants growing under the F96T/GRO/VHO. Access tube for neutron probe can be seen in container in front on left.

such as the Gro-Lux series can certainly be considered as in the right direction; however, additional efforts would also be most welcome by biologists. More thorough descriptions of presently available light sources would be of some help in their evaluation. In addition to the output data frequently available from the commercial light companies on unaged lamps, there are other measurable characteristics not normally available but also of primary importance to the environmentologist in deciding what to use—for instance (*a*) the spectral emission of lamp X as affected by ambient temperatures; and (*b*) the changes to be anticipated in spectral emission of lamp X with usage, such as those related to differential deterioration rates of the lamps' respective phosphors.

A new model tube of Gro-Lux (F96T12/GRO/VHO/WS) was being tested at the time this manuscript went to press to measure to what degree the bulb has been improved for bean or tomato growth.

Transpiration, Leaf Temperature, and Stomatal Activity of Certain Plants as Affected by CO_2 Concentration of the Air and Soil Moisture Tension

Earlier investigations (*23*) on transpiration from corn plants indicated that a relatively high percentage of the stomata remained closed under conditions expected to foster high stomatal activity. This finding posed the question of why stomatal activity was low under what were considered optimum environmental conditions. Field observations (see pp. 18–19) indicated considerably more open stomata could be expected. Further experimentation showed that the CO_2 concentration of the air in a plant's environment can drastically affect stomatal opening. Thus, the high CO_2 concentrations in the growth room (as noted in *23*, figs. 21 and 22) were responsible for low stomatal activity. Since many of the stomatal responses recorded in the aforementioned studies were within the range of external CO_2 changes in the environ-

ment, the influence of CO_2 content in air needed assessing to answer the following:

(*a*) Does cuticular transpiration of crop plants assume proportions not heretofore recognized? (*b*) How do changes in cuticular and stomatal transpiration affect the heat budget of the plant? (*c*) Of what importance to stomatal operation is soil moisture tension as opposed to CO_2 concentration of the air? Answers to these questions are paramount to man's controlling the moisture loss from plants and increasing his economy of water use.

Our knowledge of the total quantity of water transpired by plants that can be ascribed to cuticular transpiration seems to be somewhat clouded by the experimental methods employed by various researchers. Many attempts in the past to estimate cuticular transpiration appear to have had limitations that could result in low cuticular values. The inadequacies of such methods have never been completely discussed or defined. Probably the most serious error in early studies was the use of hypostomatic leaves. Transpiration from such leaves has been measured before and after coating the underside of the leaves with white vaseline or cocoa butter or both. (See Stålfelt (*39*) for extensive literature citations.) Cuticular transpiration was considered to be that which was recorded in the coated condition. In a little different experimental approach Fusser (*6*) has caused the absorption of transpired water from both hypostomatic leaf surfaces simultaneously and independently, but this upset the microenvironment.

It would appear that such techniques employing hypostomatic leaves cannot be directly compared with nonhypostomatic leaves because of several possible dissimilarities. First, differences in cuticle thickness on upper and lower surfaces were not ascertained. Also, the cuticular chemical composition of the two surfaces may be different enough so as to differentially affect the magnitude of transpiration. Comparative use of transpiration values from the two surfaces also obviously fails to consider the plumbing interior of the leaf and its possible effect on the water available for cuticular transpiration from either or both surfaces. Kamp (*12*) has postulated that the epidermal cells are more of a factor in controlling cuticular water loss than any differences in cuticular thickness. We must then ask ourselves the question: What is the normal route or routes for water molecules to follow as they pass to the gaseous phase—through and from the upper epidermis and lower epidermis, or through and from bundle parenchyma, mesophyll cells, or intercellular spaces and out the stomata? It is obvious that no one answer will usually suffice, considering all aspects of the plant in question and its environmental status.

The other method employed to some extent for assessment of the magnitude of cuticular transpiration is the weighing method. Stålfelt (*38*), Pisek and Berger (*28*), Hygen (*9*, *10*, *11*), and others consider the rates of water loss of severed leaves (transpiration decline) as being directly related to stomatal aperture and, thus, by following the transpirational loss with time, an assessment of the magnitude of cuticular and stomatal transpiration can be made. As transpiration continues, water content of severed leaves also drops. Since the leaf (severed or not) has a limited reservoir, free energy of its water decreases with time; therefore, both stomatal and cuticular and, finally, any wholly cuticular transpiration should show decline. Hygen (*10*) considered transpiration thus, but Williams and Amer (*46*) have disagreed with his assumption that transpiration from a leaf can be treated as though the vapor pressure at the evaporating surface falls in direct proportion to the water content. They consider such a postulate generally invalid since transpiration from the experimental subject, *Pelargonium*, appeared to them to be independent of leaf-water content. In turn, Williams and Amer's work must be questioned as to the relevancy of their *Pelargonium* data compared with the species Hygen used, which did not include *Pelargonium*.

Rather high cuticular transpiration values *are* recorded in the literature. Pisek and Berger (*28*) have compared cuticular transpiration of a number of diverse species with evaporation from blotter paper under average room conditions. Quite extreme values were obtained from species representing different habitats. Transpiration of *Opuntia camanchica* was as little as 0.03 percent of the evaporation from blotter paper, whereas *Impatiens noti tangare* transpired at a rate greater than 50 percent of blotter paper evaporation. Hygen (*10*) obtained cuticular transpiration values ranging from 10 to 20 percent for some mesophytes and values of 25 to 40 percent for what he describes as plants growing without moisture stress.

Our approach to the problem was to change the CO_2 concentration in growth chambers to effectively open or close stomata. We then determined transpiration when stomates were closed versus transpiration when stomates were open. Transpiration was determined by weight changes observed per unit of time. Simultaneously, leaf temperatures were assessed by thermocouples. Finally, the stomata were opened by lowering CO_2 concentrations, and stomatal activity was recorded as soil-moisture tension increased. Our hypothesis was that cuticular transpiration of crop plants is of considerable magnitude.

Materials and Methods

Five species were used—corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), sorghum (*Sorghum vulgare* Pers.), soybean (*Glycine max*

Merr.), and tomato (*Lycopersicon esculentum* Mill.)—a total of 17 lines, hybrids, and varieties (table 2). All are important agriculturally except the three single-cross corn lines and Smooth Leaf Empire cotton. The corn lines were included because they have shown distinctly different relationships between yield and soil moisture in field studies. Smooth Leaf Empire cotton was included because it contains a D_2 gene thought to increase cuticle thickness, eliminate pubescence, and decrease evaporation through the upper leaf surface.

TABLE 2.—*Transpiration and percentage stomata open on upper and lower leaf surfaces at CO_2 concentrations <250 p.p.m. and 400 to 500 p.p.m. and percent reduction in transpiration resulting from the higher CO_2 concentrations*

Crop, age of plants, and "variety"	<250 p.p.m. CO_2				400 to 500 p.p.m. CO_2			Transpiration reduction
	Transpiration	Standard deviation	Stomata open	Lowest CO_2 value maintained	Transpiration	Standard deviation	Stomata open	
	G/dm²/4 hr.		*Percent*	*P.p.m.*	*G/dm²/4 hr.*		*Percent*	*Percent*
Corn (21 days):								
Dixie 82	14.44	0.76	100	20	4.75	0.77	0	68
MP339 x MP311	12.90	.15	100	20	4.14	.56	0	68
MP305 x T101	12.57	.60	100	20	4.34	1.60	0	66
MP305 x MP307	11.85	.46	100	20	3.91	.94	0	68
Sorghum (29 days):								
RS–610	11.75	1.30	100	30	3.85	2.2	0	68
NK–210	11.50	1.30	100	30	4.92	1.7	0	58
Amak–R	11.00	.86	100	30	4.34	1.3	0	61
Tomatoes (43 days):								
Marglobe	10.43	1.30	87	115	8.24	1.10	0	21
Rutgers	9.35	1.03	83	115	6.32	.48	0	33
Marion	9.29	1.03	85	115	5.97	.51	0	36
Soybeans (36 days):								
Hampton	10.27	2.50	91	35	6.87	1.7	0	34
Hardee	8.90	1.03	91	35	4.27	1.4	0	53
Beinville	8.83	.62	92	35	5.28	1.5	0	41
Cotton (56 days):								
Smooth Leaf Empire	7.15	.62	87	40	4.75	.59	0	34
Auburn	7.03	.73	92	40	5.05	.69	3	29
Empire	3.49	.64	84	40	2.33	.83	2	34
Carolina Queen	3.11	.47	85	40	2.34	.70	2	25

General growth conditions.—A standard fertilized, Krilium-treated soil was prepared as previously described (*23*) and used for plant growth. All seeds were pregerminated in vermiculite; uniform populations were selected on the basis of radicle length and were transplanted 24 to 32 hours after sowing. Single plants were grown in 3,600 grams of Cecil sandy clay loam in tarred 92-ounce juice containers. During the preexperimental periods, soil moisture availability was kept above 50 percent but did not exceed 0.05 bar. Such soil moisture control was expected to minimize its effect on plant growth and related processes. Moisture desorption curves were developed (*30*) and used for relationships of water availability with matric suction. Experiments were performed only after fully expanded leaves had developed but before self-shading or moisture drawdown limited physical description. All populations were grown and tested in controlled environment chambers. Growth and test conditions were: 14 hours light (VHO cool white fluorescents approximately 0.5 to 0.6 cal. $cm.^{-2}$ $min.^{-1}$), temperature 25° C., relative humidity 50 to 70 percent; and 10 hours dark, temperature 20° C., and relative humidity 85 to 95 percent.

CO_2 control of stomata.—Preliminary experimentation indicated that various carbon dioxide concentrations could effectively open or close the stomata of crop plants. The response was not consistent at any one CO_2 concentration from species to species. Neither did all stomata on a leaf behave exactly the same at the same concentration of CO_2. Apertures changed with changes in CO_2 concentration; however, even more striking than changes in aperture was the finding that in a given microscopic field on a single leaf a large number of stomata can appear closed while others appear open.

Figure 6 shows the CO_2 concentrations in air as they change stomatal condition of the several varieties sampled under the conditions stated. This research was first attempted in small leaf

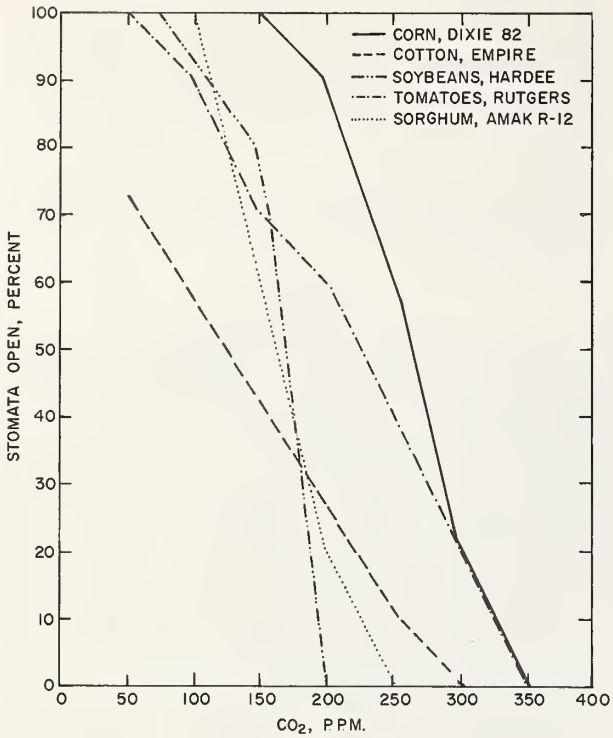

FIGURE 6.—Influence of changing CO_2 concentration in air on 10 selected stomata contained in a microscopic field. Light intensity approximately 0.5 cal. $cm.^{-2}$ $min.^{-1}$, temperature 25 ° C., relative humidity 50 to 70 percent.

chambers but was transferred to growth chambers when it was found that the leaf was frequently in delicate equilibrium with the rest of the plant. Differences between leaf chamber environment and plant environment were found to cause abnormal stomatal activity in the leaf chamber.

Face masks (fig. 7) for expiring exhaled air high in CO_2 were required to maintain low concentrations of CO_2 in the growth chamber. Both control and monitoring of CO_2 were accomplished with a Liston-Beckman infrared gas analyzer, model 15A.

Measurements of observed *cuticular and stomatal transpiration.*—Estimates of cuticular and stomatal transpiration of the species were made by weight differences on several consecutive days. All weights except plant weights and soil moisture weights remained constant. To estimate plant weight, six representative plants of each variety [6] were sacrificed the evening before the first experimental run. During the experimental period soil moisture availability was kept high (less than 0.3 bar) to minimize its effect on stomatal operation or transpiration. Plants were watered to 0.05 bar soil moisture tension the evening preceding each experimental day. Five replicates were the minimum used in any study. All varieties of a species were tested simultaneously; however, the different species were run separately. Stomatal and cuticular transpiration were measured daily over a 4-hour period under low CO_2, with the stomata open. *Observed* cuticular transpiration was also measured daily over a 4-hour period under high CO_2, with stomata visibly closed, or in some instances nearly closed. When stomata were visibly closed, the difference between the two measurements was considered to be cuticular transpiration.

Studies of soil moisture tension effects on stomatal opening under low CO_2 were started as soon after stomatal and observed cuticular transpiration measurements as practicable. For these studies the plants were watered to 0.05 bar after lights were out; then, the next morning the CO_2 was lowered to cause the stomata to open and was maintained low during the daylight hours of each experiment. The percentage of stomata open was followed microscopically during daylight hours (usually for several days) as soil moisture tension increased and the stomata shut.

The leaf areas in all studies were determined as reported for corn and sorghum (*23*) and cotton (*2*), and for tomatoes and soybeans by cutting out the shadow-cast replicas of leaves from ozalid paper and relating their weight to actual weight per unit area.

Stomatal monitoring.—The condition of the stomata on upper and lower leaf surfaces was assessed hourly by use of a special microscope (*25*). Records were kept on the number of stomata open on the upper and lower epidermis; 40 individual stomatal counts, 2 leaves per plant, 2 plants per variety were the minimum. After the counts were made and recorded, all the plants were scanned hourly to see if stomatal reaction was uniform.

Leaf temperature.—The temperature of upper and lower leaf surfaces was continually sensed by

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FIGURE 7.—Monitoring stomatal activity to determine effects of CO_2 concentration or soil moisture tension. Face mask was used to maintain low CO_2.

[6] "Variety" is used in a loose sense hereafter to indicate lines, hybrids, or varieties.

small thermocouples (*23*) placed in intimate contact with the cuticle and held by small pieces of masking tape. Four thermocouples per plant, with one plant representing each variety, were used.

Results

Transpiration.—The observed cuticular transpiration values obtained in these studies (table 2) appear quite high when compared with the average value of 90 percent so often quoted as typical of transpiration through stomata (*13*, *15*). If we accept low cuticular values as normal, then a rather large error must exist in our analysis. Either a low number of stomata open under the low CO_2 or incomplete closure of stomata under high CO_2 could effectively increase the proportion of transpiration considered cuticular in this study. The recent report of Ting and Loomis (*41*), as well, as the older work of Stålfelt (*38*) and Hygen and Midgaard (*11*), indicates that the degree of opening as reflected in size of the stomatal pore above several microns may be of minor importance in determining the magnitude of transpiration. Most important is whether the stomata are open at all. Table 2 summarizes the observed stomatal condition of the species during the experimental periods. Stomata of the crop plants exclusive of cotton were observed to be closed under the high CO_2; however, none of the dicotyledonous plants had all stomata visibly open under low CO_2 (see table 2). The variations in transpiration as expressed by the rather high standard deviations associated with the transpiration measurements are noteworthy; possibly they indicate the stomatal condition of our plants was poorly defined because we were unable to ascertain complete closure or because the sampling of stomata was inadequate. Successive runs with several other populations of corn plants indicated that the listed values of transpiration under the same experimental conditions were reproducible. The standard deviations in table 2 were used to set limits for the realistic ranges of transpiration described in table 3.

For those species indicating complete visual stomatal closure the question arises whether a hermetic seal existed at the interface of the guard cells knowing the resolution at the magnification used ($\sim 2\ \mu$) was insufficient to detect complete closure. For the present we do not have a completely satisfactory method for measuring either the stomatal seal or improving our microscopic potential. Hygen's approach of studying the change in transpiration with time as related to stomatal closure (*9*, *10*, *11*) is probably the best approach presently available, but his method has been limited to severed plant parts. In our future studies his method will be employed with whole plants to further clarify transpiration-stomatal-CO_2 interactions.

TABLE 3.—*Range of transpiration after stomatal closure expressed as percentages of total transpiration*

[Values based on standard deviations of table 2]

Crop and "variety"	"Observed" cuticular		Cuticular + stomatal		Range
	Low	High	Low	High	
	G/dm²	*G/dm²*	*G/dm²*	*G/dm²*	*Percent*
Corn:					
Dixie 82	3. 99	5. 51	13. 67	15. 21	26–40
MP339×MP311	3. 99	4. 29	12. 34	13. 56	30–35
MP305×T101	3. 74	4. 94	10. 97	14. 17	26–45
MP305×MP307	3. 45	4. 37	10. 91	12. 79	27–40
Sorghum:					
RS–610	2. 55	5. 15	9. 59	13. 95	18–53
NK–210	3. 62	6. 22	9. 80	13. 20	27–63
Amak–R12	3. 48	5. 20	9. 70	12. 30	28–54
Tomatoes:					
Marglobe	6. 94	9. 54	9. 33	11. 53	60–90
Rutgers	5. 29	7. 35	8. 87	9. 83	54–92
Marion	4. 94	7. 00	8. 78	9. 80	50–80
Soybeans:					
Hampton	4. 37	9. 37	8. 57	11. 97	37–89
Hardee	3. 24	5. 30	7. 50	10. 30	32–71
Beinville	4. 66	5. 90	7. 33	10. 33	45–81
Cotton:					
Smooth Leaf					
Empire	4. 13	5. 37	6. 56	7. 74	53–82
Auburn 56	4. 32	5. 78	6. 34	7. 72	56–91
Empire	1. 69	2. 97	2. 66	4. 32	39–90
Carolina Queen	1. 87	2. 81	2. 41	3. 81	49–85

Leaf temperature.—Changes in leaf temperature with changes in stomatal condition are reflected in table 4. In general, leaf temperature increased several degrees for corn, sorghum, tomato, and soybean plants when stomatal transpiration was minimized. Cotton leaf temperature did not change significantly. Optimum soil moisture in this phase of the study probably had some bearing on the leaf temperatures recorded.

Soil moisture tension and stomatal operation.—Figure 8 depicts how stomata opened by low concentrations of CO_2 respond to increases in soil moisture tension. The responses observed differ among species and among some varieties of the same species.

Discussion

If the stomata observed in our studies were completely closed, the data indicate that high cuticular rates may also occur in several of the crop plants we tested. The values must be interpreted with due caution. They are presently indicative of transpirational changes brought about by changes in CO_2 levels for the conditions of growth and experimentation employed. Our hypothesis still re-

TABLE 4.—*Radiation impinging (R), temperature (LT) and energy dissipated as latent heat (E_L) of five species of leaves at high and low concentrations of CO_2 in the atmosphere*

[Latent heat exchange as based on average transpiration values in table 2]

Crop and variety	R[1]	High CO_2		Low CO_2		ΔT	ΔE_L
		LT	E_L	LT	E_L		
	Cal.[2]	*°C.*	*Cal.*[2]	*°C.*	*Cal.*[2]		
Corn:							
Dixie 82	0. 50	28. 7	0. 12	25. 3	0. 35	3. 4	0. 23
MP339× MP311	. 50	29. 2	. 10	25. 9	. 31	3. 3	. 21
MP305× T101	. 50	28. 1	. 11	24. 8	. 30	3. 3	. 19
MP305× MP307	. 50	27. 6	. 10	24. 0	. 29	3. 6	. 19
Sorghum:							
RS-610	. 50	30. 4	. 09	26. 4	. 28	4. 0	. 19
NK-210	. 50	29. 8	. 12	25. 7	. 28	4. 1	. 16
Amak-R12	. 50	28. 6	. 11	26. 5	. 27	2. 1	. 16
Tomatoes:							
Marglobe	. 50	29. 1	. 20	25. 6	. 25	3. 5	. 05
Rutgers	. 50	25. 4	. 15	24. 0	. 23	1. 4	. 08
Marion	. 50	29. 2	. 14	26. 8	. 22	2. 4	. 08
Soybeans:							
Hampton	. 60	30. 6	. 17	25. 5	. 25	5. 1	. 08
Hardee	. 60	29. 8	. 10	25. 1	. 22	4. 7	. 12
Beinville	. 60	28. 9	. 13	24. 6	. 21	4. 3	. 08
Cotton:							
Smooth Leaf Empire	. 58	26. 5	. 12	24. 8	. 17	1. 7	. 05
Auburn 56	. 58	25. 2	. 12	24. 7	. 17	. 5	. 05
Empire	. 58	24. 7	. 06	23. 9	. 08	. 8	. 02
Carolina Queen	. 58	23. 9	. 06	24. 6	. 08	−. 7	. 01

[1] Measured with a Beckman-Whitley Model H 188-01 radiometer.
[2] Cal. cm.$^{-2}$ min.$^{-1}$.

mains to be proved or disproved, since the state of stomatal opening below 2μ was not defined.

However, in light of some of the earlier discussion, it is obviously imperative that we reassess our physical description of gaseous transfer by leaves. The stomatal pore has too long been emphasized as the portal of entry and exit of water and CO_2 molecules. In studies of impedance (rather than the term resistance because of vectors involved) to water movement from any leaf we should consider both the stomatal and cuticular routes. Both the air layer adjacent to evaporative cell surfaces and the evaporative cell surfaces themselves are important components in the vaporization process; however, pathways leading to these evaporative surfaces are equally important. Wylie's (*49*) and Armacost's (*1*) work indicated that vein extensions and the epidermis supplement water transfer by the mesophyll and in the leaf may be the primary route of water transfer from veins to evaporative surfaces. The work of Roberts et al. (*31*) also suggests that the route of water movement in itself involves such vein extensions along pectinaceous paths. Our knowledge in this area is scant. In the plant there exists an impedance to water movement up to and including the leaf surface and stomatal pore. Such impedance in the leaf is quite complex; it includes such factors as the cell wall matrix, the free energy status of water available for transpiration, and the chemical and physical constitution and state of cuticle and suberin, and their underlying layers offering resistance to flow. Also, the involvement of the protoplasm, especially ectodesmata (*5*) in the movement of water eventually transpired, is of some presently undefined importance; albeit dealing with protoplasmic water in itself, its availability is ill defined. The efficiency of flow (diffusion of water vapor) from mesophyll walls through the stomatal pore is probably not as simple in all species as Bange (*3*) describes for *Zebrina pendula* Schuizl.

If the intercellular air were saturated with water vapor at all times and in all places, the vapor transfer phenomenon from mesophyll cells through stomatal pores might be treated simply; however, we have no assurance that this is the case. Dynamic changes in the plant's environment alone will affect the microconditions, both external and internal to the leaf. Thus, we can interpret the hourly fluctuations in stomatal opening (*35*, *37*) induced by environmental demand, as reflecting the inability of water to move from the soil to the plant to continuously meet such demand.

From this and our previous work, the authors recognize that the major barrier to an accurate assessment of the impedance at the leaf surface when stomata are not fully opened involves the lack of constancy in stomatal response. Throughout these studies (*26*, *23*) we have continued to report stomatal operation as the percentage of stomata open. When some stomata can be completely open and some completely closed on the same leaf, (see p. 36 and fig. 28 of *23*), or when 50 percent of the stomata assayed appear closed, or when their activity is oscillatory, then average stomatal aperture is somewhat ambiguous.

There is also relevance in these studies to CO_2 passage through the leaf. The CO_2, which is essential for photosynthesis, must solubilize when diffusing through aqueous and possibly lipoidal pathways before entry into the chloroplasts. CO_2 probably solubilizes under certain conditions via the cuticle, and, thence, diffuses to palisade and mesophyll cells. Such diffusion would be complementary to diffusion into substomatal chambers and, thence, to palisade or mesophyll cells. Under

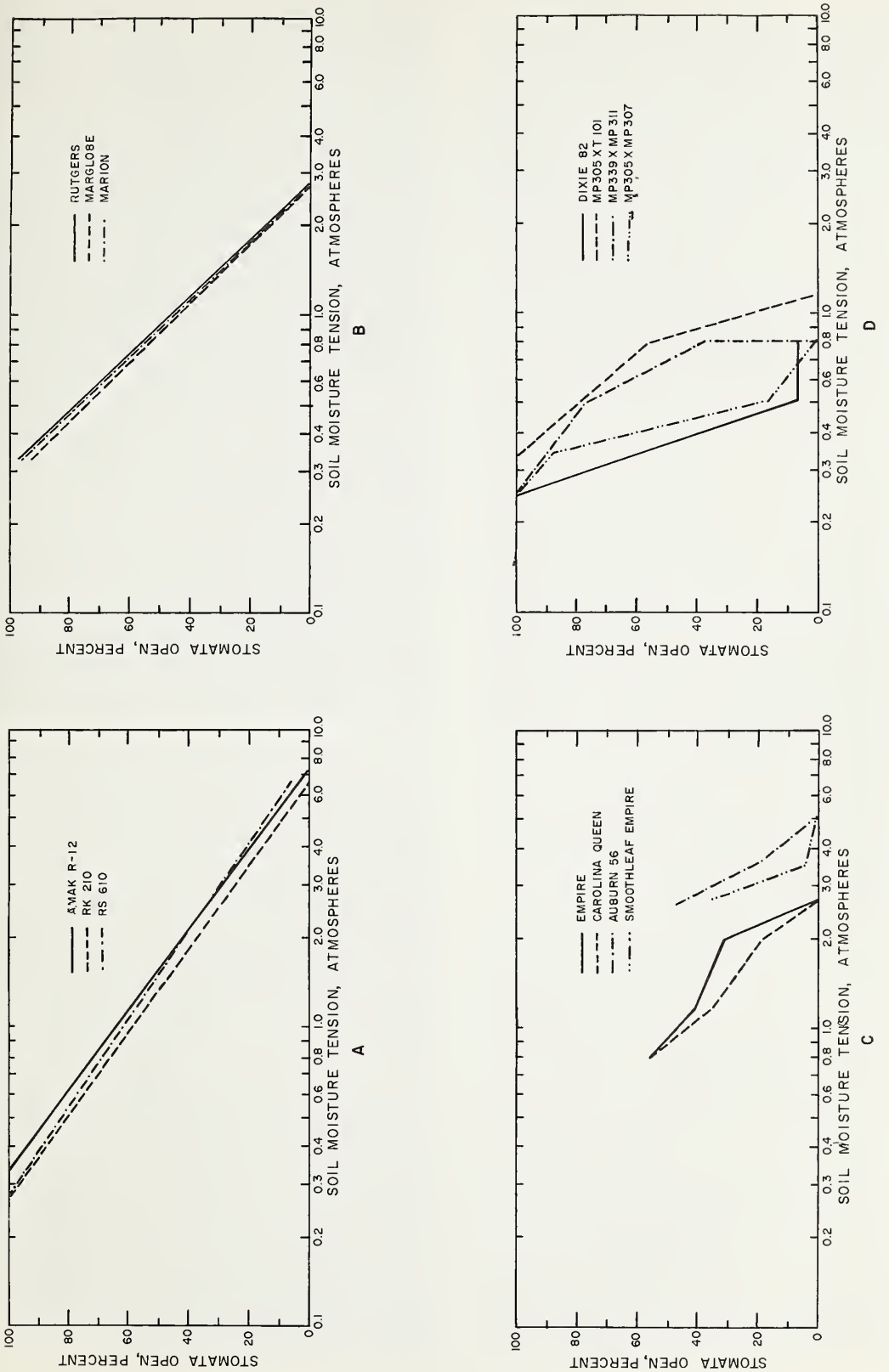


FIGURE 8.—Stomatal activity under low CO_2 concentrations in air as related to increasing soil moisture tension—(A) sorghum, (B) tomatoes, (C) cotton, and (D) corn.

those conditions where stomata are completely closed it would be the only pathway of CO_2 diffusion. Involvement of this route could help explain why under enriched CO_2 supply (*7*, *11*, *26*) plants may increase their assimilation rate even when stomata appear to close and transpiration markedly decreases. Differences in diffusive resistance of the cuticle between species could also explain the variation in efficiencies found between species utilizing the same concentration of CO_2 (*8*) for photosynthesis.

Table 4 summarizes leaf temperature changes of the individual varieties as well as energy dissipated by transpiration. Such data are almost nonexistent (*29*). Six degrees centigrade (RS610) above the ambient temperature of 25° C. was the highest recorded in these studies. In general, leaf temperatures increased several degrees for corn, sorghum, tomato, and soybean plants when stomatal transpiration was reduced. Recorded leaf temperatures of the corn leaves are essentially the same as those already reported (*23*) for corn grown under similar environmental conditions and total radiant energy, but under incandescent lights. The most striking difference found among the species listed in table 4 concerns the leaf temperature of cotton plants. Although cotton leaves had the lowest transpiration of all the species tested, and thus the least energy dissipated as latent heat, they had a remarkable tendency to remain either below ambient temperature or at the most a degree and a half above. Thus, the transpiration of cotton leaves does not account in any large measure for the dissipation of impinging radiation. Cotton's low leaf temperature may result from either or both a high capacity of the leaves for convective and reradiative loss or low long-wave interception, such as would result from a high ability for reflection or transmittance, or both.

Figure 8 shows that when the CO_2 of the atmosphere was kept low to effectively open the stomata, increasing soil moisture stress eventually reached a point that offset photoactive opening (*26*) and brought about complete visible closure. For corn, sorghum, and tomatoes the first response was near 0.3 bar moisture tension. Other than this initial effect, the slope and intercepts of the curves are quite different. The stomata of the different tomato varieties and sorghum hybrids responded within species in a remarkably uniform manner. Tomato stomata closed at a soil moisture tension several bars lower than sorghum stomata. Corn stomata closed at significantly lower soil moisture tensions than any of the other species.

The data also indicate that variation existed in the stomatal response between the variety and lines. There may be an important correlation between the field observation that Dixie 82 is drought resistant and the data in figure 8 indicating that its stomata close at lower soil moisture tensions than the other lines tested. Analysis of the extent of root systems in these studies, as indicated by their fresh and dry weight, showed Dixie 82 had a much smaller root system. It is possible that a smaller root system, especially in the shallow soils found in the southern Piedmont, would be more conservative of soil moisture. Such a root system could not absorb water at the same rate (to meet evaporative demand) as a more extensive root system. The ultimate effect would be an earlier closure of stomata, as seen in figure 8, and from there on a more conservative use of soil water by prolonging the period of availability. Complete stomatal closure of Dixie 82 was not observed until soil moisture tension reached the same value as that attained with the other varieties. In periods of high soil moisture availability Dixie 82 would develop at maximum efficiency. This hypothesis is opposite from what one would expect for the adaptation of corn plants to a deep soil profile in which a larger root system extending to greater depths could tap a greater soil water reservoir.

At the outset of the studies on soil moisture tension cotton stomata were never completely opened by low CO_2. Recent experimentation has shown complete opening is possible at higher radiant energy values and different spectral quality.

The curves in figure 8 show that Empire and Carolina Queen, and also Auburn 56 and Smooth Leaf Empire, tend to parallel each other in their soil moisture stomatal response. Their cuticular and stomatal transpiration (table 2) also show the same tendencies for grouping. Further experimentation is necessary to prove or disprove that such differences are relevant to soil moisture conservation. The high rate of transpiration per unit of leaf surface of Smooth Leaf Empire (table 2) does introduce an element of uncertainty that its D_2 gene improves its drought resistance as stated earlier.

In these studies the ability of low CO_2 to maintain stomata in an open position has been shown to be of secondary importance to that of increasing soil moisture tension and is in agreement with Stålfelt's recent findings (*40*). Soil moisture tensions greater than 1 bar have a drastic effect on guard cell activity, as indicated by our studies on corn and sorghum (*26*). The number of stomata open continuously decreased as the soil moisture tension increased, with the magnitude of the effect of this increase determined by air vapor pressure difference and radiation level.

GUARD CELL ACTION

Protoplasmic Streaming and Guard Cell Operation

That guard cell cytoplasm undergoes cyclosis has been reported by both Weber (*44*) and Sinke (*34*). This protoplasmic activity has been used by several workers to ascertain whether guard cells were alive or dead; however, very little effort has been put forth to characterize the streaming phenomenon. Weber states that streaming is characteristic of closed stomata (in *Vicia faba*); with opening, the phenomenon is reduced to a local sliding motion, finally, ceasing, or on rare occasions exhibiting local jerky sliding motions in the fully opened stomate.

In cursory examinations cyclosis in guard cells of several species (including *Vicia faba*) was detected; however, its association, as depicted by Weber, with the closed condition was not absolute. In attempts to document cyclosis in guard cells of *Vicia faba* on movie film, working shortly after dark gave us consistently a few subjects to record.

Our studies on cyclosis were initiated to find out if streaming is correlated with the peculiarities of guard cell activity, such as stomatal opening and closing, or is just another indication of a living cell. Only the first experiment will be discussed here; others continue to be performed and their descriptions will follow in subsequent reports.

Procedure

The first series of experiments was designed to find out what, if any, correlation exists between guard cell streaming and time of day. A vigorously growing population of *Vicia faba* (horsebean), *Rheum rhaponticum* (rhubarb), *Cyclamen indicum* (cyclamen), and *Antirrhinum majus* (snapdragon) in individual 6-inch pots were transferred from the greenhouse to a growth chamber a week before the study of streaming and guard cell operation. In the growth chamber a 12-hour photoperiod and 20° day and 15° night temperatures were standard. Light was provided by cool white fluorescents (0.5 cal. cm.$^{-2}$ min.$^{-1}$) from 6 a.m. to 6 p.m.

Beginning the day that the plants were placed in the growth chamber, daily observations were made microscopically to determine whether or not stomata were open. At least 10 stomata of two selected (tagged) mature leaves of each species were observed each time. Later, when both opening and streaming were being assessed, similar areas on untagged leaves were observed and if the stomata were essentially in the same condition as those monitored on tagged leaves, the areas observed on the untagged leaves were stripped as quickly as possible, placed in water, and checked for streaming under oil immersion. This method of consistently observing the same tagged leaves provided a check on changes in stomatal activity that might occur during growth. Checks were made on all species just before and immediately after "lights on" in the morning, at noon, and in the evening before and right after darkness. The first experimental period lasted 9 days. The light was then reduced to 0.05 cal. cm.$^{-2}$ min.$^{-1}$ for 6 days of observation.

Results and Discussion

For several days preceding and including the day of transfer from greenhouse to growth chamber, March 26, 1963, the sky was overcast, there were occasional showers, and, in general, light intensity was low. A low number of open stomata were found the first few days after the plants were transferred to the growth chamber. As can be seen in the March 28 observations contained in table 5, more *Vicia faba* stomata are open during the day than during the night. A similar cycle, but at an almost imperceptible level of activity, occurred during the same period on snapdragon and cyclamen leaves. After 7 days in the growth chamber, the number of stomata opening increased considerably (compare, in table 5, 3–28 with 4–4 and then 4–10). *Vicia faba* stomata apparently reached photoactive saturation, with opening maintained both day and night. Cyclamen and snapdragon also showed considerable increase in the number of stomata visibly open both day and night with increased number of days in the growth chamber. The tendency for rhubarb to show considerable night opening (4–10) is not consistent with our previous report (*23*); however, the activity at the lower light level (4–17) is. The discrepancy may be because of the different light sources used (incandescent in the earlier study versus fluorescent in these) or differences in CO_2 availability between the two studies. Infrared gas analysis has shown there is a tendency for CO_2 to remain at external levels or lower in growth chambers that contain a photosynthesizing population. As already explained (see p. 5), growth room studies have been affected by CO_2 concentrations usually exceeding outside air values, thus causing in some species less stomatal activity than would be normal.

The tendency for carryover of open stomata into the dark periods was related to the light intensity to which the plants had been subjected. It was deduced that the photoactive phase of opening was light saturated. To test this hypothesis, lights were reduced to 0.05 cal. cm.$^{-2}$ min.$^{-1}$ on April 15. This resulted in a drastic reduction in the percentage of stomata open by April 17 during both the night and day (table 5). More definite

TABLE 5.—*Percentage of stomata open in growth chamber* [1]

[1963]

Species and date	5:30 a.m.	Light						6:30 p.m.	Midnight
		6:00 a.m.	6:30 a.m.	9:30 a.m.	10:30 a.m.	Noon	5:30 p.m.		
Vicia faba:	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
3–28	0	30	90	------	------	100	90	0	------
4–4	90	------	90	100	100	100	100	90	------
4–10	90	------	90	------	------	90	100	80	80
4–17	0	------	10	------	------	30	40	20	0
Antirrhinum majus:									
3–28	0	0	0	------	------	20	5	0	------
4–4	10	------	20	60	70	80	80	70	------
4–10	20	------	70	------	------	100	80	40	0
4–17	0	------	0	------	------	0	10	10	0
Cyclamen indicum:									
3–28	0	5	10	------	------	5	5	0	------
4–4	0	------	30	30	------	50	60	50	------
4–10	10	------	70	------	------	80	40	30	0
4–17	0	------	10	------	------	10	20	20	0
Rheum rhaponticum:									
4–10	100	------	100	------	------	100	100	100	0
4–17	10	------	10	------	------	80	80	70	0

[1] Conditions in growth chamber are described on page 13.

patterns reappeared of opening in light and closing in dark. These patterns somewhat repeated those found after the original transfer from the greenhouse to the growth chamber, but in contrast to the earlier findings, stomata remained open after dark.

Very little cyclosis in guard cells was found any time during these studies. Streaming appeared to take place only when the stomata were closed and then mainly during daylight hours. At the higher radiant energy value the number of guard cells showing streaming at times appeared inversely related to the number of stomata open; but at the lower energy level, streaming was observed in 10 percent of the *Vicia faba* cells only at midnight. No streaming was observed at any time with the other three species. This study strengthened our belief that if cyclosis was associated with guard cell movement, it was not as readily apparent as Weber (*44*) had indicated. More detailed hour-by-hour studies are contemplated on guard cell cyclosis and opening movements of stomata.

During these studies it was discovered that frequently stomata observed as closed *in situ* opened when the epidermis was peeled and placed on water. This finding has considerable relevance to our cellular studies and will be discussed in future reports.

From the observed stomatal response to changes in light quantity, we can hypothesize a relationship between cool-weather crops and their response to radiant energy. These studies indicate that both heat and drought tolerance of the test species are correlated with their stomatal response and plastid number.

Table 5 shows that the species *Vicia faba* and *Rheum rhaponticum* had the highest percentage of stomata open at both high- and low-light values. The plastid number and size of guard cells in these two species appeared to be significantly greater than in *Cyclamen indicum* and *Antirrhinum majus*. Of the four species studied, the snapdragon has the smallest number of plastids per guard cell and is probably the most heat- and drought-tolerant. Rhubarb and cyclamen are somewhat intermediate between cool- and warm-weather crops; horsebean is thought to be a cool-weather crop intolerant of warm weather. Any classification of plant species into warm- and cool-season crops is very general and will have exceptions.

Effective radiation levels are normally greater during warm seasons than during cool seasons because of the angle of incident radiation and day length. In these studies guard cells of the cool-season crops evidently became saturated by light, thus the photoactive and hydroactive balance, which a plant must maintain with its environment, was upset. These studies indicate that a cool-season plant such as *Vicia faba* grown under an intermediate temperature and high total daily light (360 cal. $cm.^{-2}$ day) ceases to have normal stomatal reactions. Under natural conditions this combination of temperature and light could be lethal for *Vicia faba*. With all stomata open night and day, larger quantities of water would be

transpired, thus upsetting the total water balance of the plant and certainly reducing potential water recharge of tissues.

Much more research is necessary to prove or disprove the importance of light saturation of guard cells in any plant's ecological relationship; however, it is evident that normal metabolic processes (including stomatal action) may be altered by changing the temperature and light environment of the plant. Experimenters should give more consideration to environmental conditions when they are using controlled facilities. The optimum normal environment of the plant in the field should serve as a base for experimental conditions.

EFFECTS OF CERTAIN CHEMICALS ON TRANSPIRATION

Atrazine

In agricultural production at least 50 percent of the water that passes from the soil to the atmosphere is transpired. However, transpiration does not appear to be a biologically efficient process since less than 5 percent of the soil water absorbed is incorporated in the constitution of the plant. The potential, therefore, is thought to exist of markedly reducing transpiration by physical barriers such as wax, latex, or plastic coatings; by enzymatic control of guard cells; or by plant breeding, incorporating those morphological, physiological, and biochemical properties responsible for efficient soil moisture usage.

Published results from greenhouse work with Atrazine suggest that the compound as presently applied for weed control may also increase efficiency of water use. Smith and Buckholtz (*36*) reported reductions in transpiration of 40 percent by corn and 65 percent by soybean plants 6 hours after additions of 20 p.p.m. of Atrazine to their nutrient solutions. Reduction in transpiration was found in soil-grown plants as well as those grown in solution. Foliar applications were also effective. Wills and Davis (*47*) found a reduction in transpiration rate of whole plants and excised shoots of corn, cotton, and soybeans after 10 or 25 p.p.m. of Atrazine was added to the culture solution. The effect of the Atrazine was considered to be that of closing stomata. With such basic information at hand, it appeared a test for Atrazine effects should be made under field conditions. The field plan included an evaluation of water usage, yield, and stomatal operation of Atrazine- and non-Atrazine-treated corn under irrigated and simulated dryland conditions.

Experimental Procedure

Treatments.—Six treatments involving two soil moisture tension regimes, two corn varieties, and Atrazine versus no Atrazine were employed. They consisted of a modified split-split plot experimental design with moisture regime as the main plot, corn variety as the split plot, and Atrazine as the split-split plot replicated four times. All data were treated by factorial analysis of variance by computers under the direction of the University of Georgia Statistical Laboratory personnel.

An access tube was placed in the middle of each plot, facilitating assessment by the neutron probe method (*43*) of changes in available soil moisture during the growing season. Half of the plots received supplementary irrigation during the growing season. Water applied at any one time varied from 1 to 2 inches and was considered sufficient to lower the soil moisture tension in the top 24 inches to approximately one third atmosphere. The other plots were covered with plastic to prevent soil moisture recharge subsequent to saturation of the profile after planting; thus a droughty condition was assured.

Cultural practice.—Before planting, 84 pounds per acre of N, 73 pounds per acre of P, and 139 pounds per acre of K were broadcast and harrowed in. Atrazine at the rate of 6 pounds per acre (80W) was applied as a broadcast spray to subplots immediately after planting. After spraying, one-half inch of water was applied to all plots, and 4 days later 1 inch more was added to improve the action of Atrazine. Subplots were 25 by 25 feet on Cecil sandy loam. Eight rows of *Zea mays*, either Dixie 82 (a double-cross hybrid) or Hastings open pollinated, were planted in 40-inch rows in each plot May 17, 1962. Thirteen days after planting, seedlings were thinned to one plant per foot of row length. Two days later the soil on all nonirrigated plots was covered completely with transparent plastic and the plastic covered with one-quarter inch of soil so that ground reflectivity of the irrigated and nonirrigated plots was comparable. Noncropped plastic-covered and fallowed plots 50 by 50 feet were maintained so that subsurface water changes could be assessed as the season progressed.

Data recorded.—Soil moisture content in the increments of the profile (depths) as indicated in table 6 was determined once each week until 2 weeks before tasseling, after which it was measured twice weekly. Moisture determinations were also made the second day following irrigation or rain. Figure 9 shows the representative soil moisture desorption curves developed using the pressure membrane and plate technique (*30*). Variations of water content shown in table 6 are primarily due to differences in soil texture and possibly soil structure.

TABLE 6.—*Moisture content of plastic-covered and irrigated soil at specified depths at beginning (6/4/62) of measuring period* [1]

Treatment and species of plant	Measured at depths of—					
	0 to 9 inches	9 to 15 inches	15 to 23 inches	23 to 33 inches	33 to 45 inches	45 to 57 inches
PLASTIC COVERED						
Dixie 82:	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
With Atrazine	1. 42	1. 51	2. 60	3. 56	4. 19	4. 23
No Atrazine	1. 52	1. 58	2. 75	3. 44	3. 66	3. 85
Hastings open pollinated:						
With Atrazine	1. 64	1. 52	2. 42	3. 51	4. 36	4. 32
No Atrazine	1. 59	1. 39	2. 26	3. 54	4. 21	4. 14
IRRIGATED						
Dixie 82:						
With Atrazine	1. 55	1. 57	2. 68	3. 64	4. 19	4. 16
No Atrazine	1. 49	1. 49	2. 68	3. 73	4. 34	4. 20
Hastings open pollinated:						
With Atrazine	1. 58	1. 68	2. 73	3. 64	4. 08	4. 17
No Atrazine	1. 67	1. 78	2. 89	3. 53	3. 98	3. 95

[1] No significant difference exists between moisture contents listed at any one depth.

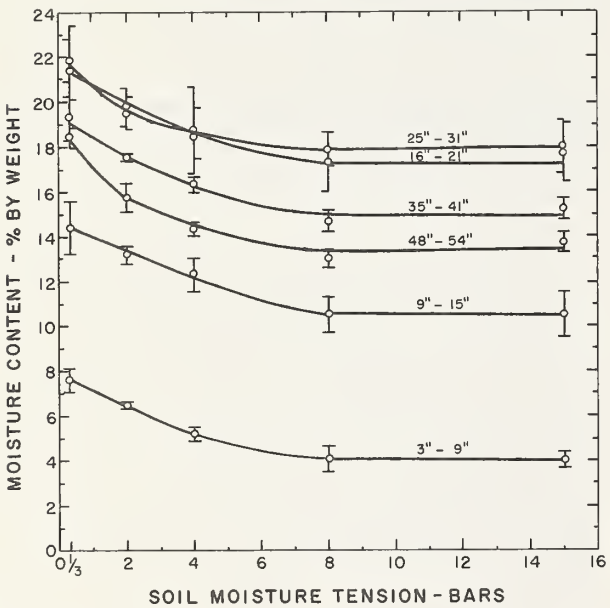


FIGURE 9.—Soil moisture desorption curves for indicated levels of soil profile. Each point represents four pressure membrane or plate determinations. A composite sample of soil was used from four different locations at the test site for each determination. Vertical lines in graphs represent standard error of the mean.

Rainfall and irrigation were recorded, thus allowing for an assessment of total water input for each plot.

Stomatal activity was recorded as the percentage of stomata open and was monitored on selected cloud-free or nearly cloud-free days throughout the growing season with the stomata viewer (*25*). The percentage of stomata open on upper and lower surfaces of the third and fourth youngest leaf midway between the tip and base was checked on the same two representative plants from each subplot throughout the season. These leaves were chosen because they were not shaded and previous experimentation (*23*) indicated all stomata on such leaves are operable.

Corn grain and aboveground dry matter yields were measured at the end of the season. When it was noted that drought was affecting the initiation of silking, counts of the number of plants silking, as related to treatment and age of the population, were made during the latter part of the season.

Results and Discussion

The heterogeneity of the soil layers made it impossible to relate neutron probe measurements to small changes in available water or soil moisture tension. Such heterogeneity is exemplified in the lack of overlapping of most of the moisture desorption curves (fig. 9). Large variations were also found in bulk density values determined at the various levels. This was both surprising and discouraging, since the area selected for the studies was considered to be one of the most geophysically uniform available for such experimentation in the Piedmont. Interpretations of water use were based, therefore, on moisture content changes both in the overall soil profile and in individual segments of the profile.

An analysis of variance on average water content of replications at the beginning of neutron probe measurements (table 6) indicated that at the age of 18 days all corn plants were under

similar soil moisture conditions at each level of the soil profile sampled. Again, at the end of the season (table 7), no significant differences were found in the soil moisture remaining at any one level of measurement between any treatments. As expected, the irrigated corn consumed significantly more water than the nonirrigated corn (table 8). Irrigated plots received 13.71 inches more moisture than the plastic-covered plots—8.96 inches of rainfall and 4.75 inches of irrigation water.

TABLE 7.—*Moisture content of plastic-covered and irrigated soil at specified depths at end of season (9/3/62)*[1]

Treatment and species of plant	Measured at depths of—					
	0 to 9 inches	9 to 15 inches	15 to 23 inches	23 to 33 inches	33 to 45 inches	45 to 57 inches
PLASTIC COVERED						
Dixie 82:	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
With Atrazine	0.74	1.14	2.23	3.19	3.80	3.78
No Atrazine	.82	1.18	2.34	2.97	3.13	3.40
Hastings open pollinated:						
With Atrazine	.89	1.11	1.95	2.94	3.83	3.77
No Atrazine	.72	.89	1.70	2.96	3.58	3.52
IRRIGATED						
Dixie 82:						
With Atrazine	.76	1.15	2.17	3.12	3.74	3.85
No Atrazine	.75	1.08	2.18	3.23	3.93	3.75
Hastings open pollinated:						
With Atrazine	.83	1.26	2.19	3.12	3.58	3.68
No Atrazine	.92	1.38	2.37	2.92	3.35	3.31

[1] No significant difference exists between moisture contents listed at any one depth.

TABLE 8.—*Seasonal water use on irrigated and plastic-covered plots, yield of corn, and efficiency of water use as shown by the transpiration coefficient*

Treatment and species of plant	Water used from depths of—						Total water used, 0 to 57 in. + irrig. & rainfall		Yield of corn per plot	Transpiration coefficient [1]	
	0–9 in.	9–15 in.	15–23 in.	23–33 in.	33–45 in.	45–57 in.	Total	Per plot		Grain	Dry matter
PLASTIC COVERED											
Dixie 82:	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*	*Pounds*	*Pounds*		
With Atrazine	0.68	0.37	0.37	0.37	0.39	0.45	2.63	8,548	24	356	74
No Atrazine	.70	.40	.41	.47	.53	.45	2.96	9,620	28	343	85
Hastings open pollinated:											
With Atrazine	.75	.41	.47	.57	.53	.55	3.28	10,660	12	888	94
No Atrazine	.87	.50	.56	.58	.63	.62	3.76	12,220	15	814	99
IRRIGATED [2]											
Dixie 82:											
With Atrazine	.79	.42	.51	.52	.45	.31	16.71	54,308	84	647	172
No Atrazine	.74	.41	.50	.50	.41	.45	16.72	54,340	83	654	171
Hastings open pollinated:											
With Atrazine	.75	.42	.54	.52	.50	.49	16.93	55,023	58	948	222
No Atrazine	.75	.40	.52	.61	.63	.64	17.29	56,095	65	863	222

[1] Pounds of water to produce 1 pound of grain or aboveground dry matter.

[2] On irrigated plots rainfall and irrigation were considered to be 100 percent effective and, therefore, added to moisture content changes, which introduces the undefined error of soil evaporative loss and deep percolation. Because the fallowed plot was always directly subject to total incoming radiation, its changes were not considered to be indicative of evaporation from the soil surface under cropped conditions.

Corn in the Atrazine-treated plots did not show a significant increase in yield (table 9) over non-Atrazine-treated plots under equal moisture availability nor more efficient use of water (table 8). A very significant increase in yield and, thus, water use efficiency by the hybrid was found, as determined by pounds of water used per pound of grain yield. Differences in net production, as measured in tons of dry matter per acre (table 9), are only significant between irrigated and droughty conditions.

Although the percentage of stomata open during any of these studies was not significantly affected by the Atrazine treatment, highly significant differences between activity on irrigated and non-irrigated plots were found. The change in stomatal activity throughout the day and season was also very pronounced. In general, the greatest number of open stomata was found in early morning (fig. 10). The decrease in number of visibly open stomata is probably associated with increased plant water stress bringing about hydroactive [7] closure as the day progressed.

The decrease in stomatal activity became more pronounced on the plastic-covered plots as the season progressed and the soil became increasingly drier. Figure 11 shows the increase in stomatal activity on June 27 as related to activity on June 26. A 2-inch rain occurred late in the afternoon of the 26th; therefore, the increased percentage of stomata open the following day is attributed to soil moisture recharge. A very low level of activity was noted the same day on the plastic-covered plots where soil moisture was not recharged. Subsequent observations indicated that there was a permanent cessation of stomatal opening on plants in the plastic-covered plots.

Not all of the progressive decrease in stomatal activity (fig. 12) as the season advanced could be

[7] See page 18 of (*26*) for discussion of this term.

TABLE 9.—*Yields of corn and stover on irrigated and plastic-covered plots* [1]

Treatment and species of crop plants	Grain yield per acre	Stover production per acre
PLASTIC COVERED		
Dixie 82:	*Bushels*	*Tons*
With Atrazine	30	3.9
No Atrazine	35	3.8
Hastings open pollinated:		
With Atrazine	15	3.4
No Atrazine	19	3.6
IRRIGATED		
Dixie 82:		
With Atrazine	105	7.2
No Atrazine	104	7.3
Hastings open pollinated:		
With Atrazine	73	6.0
No Atrazine	81	5.9

[1] Highly significant differences in yield and dry matter exist only between plastic-covered and irrigated treatments, or between hybrid and open-pollinated corn in either plastic-covered or irrigated plots.

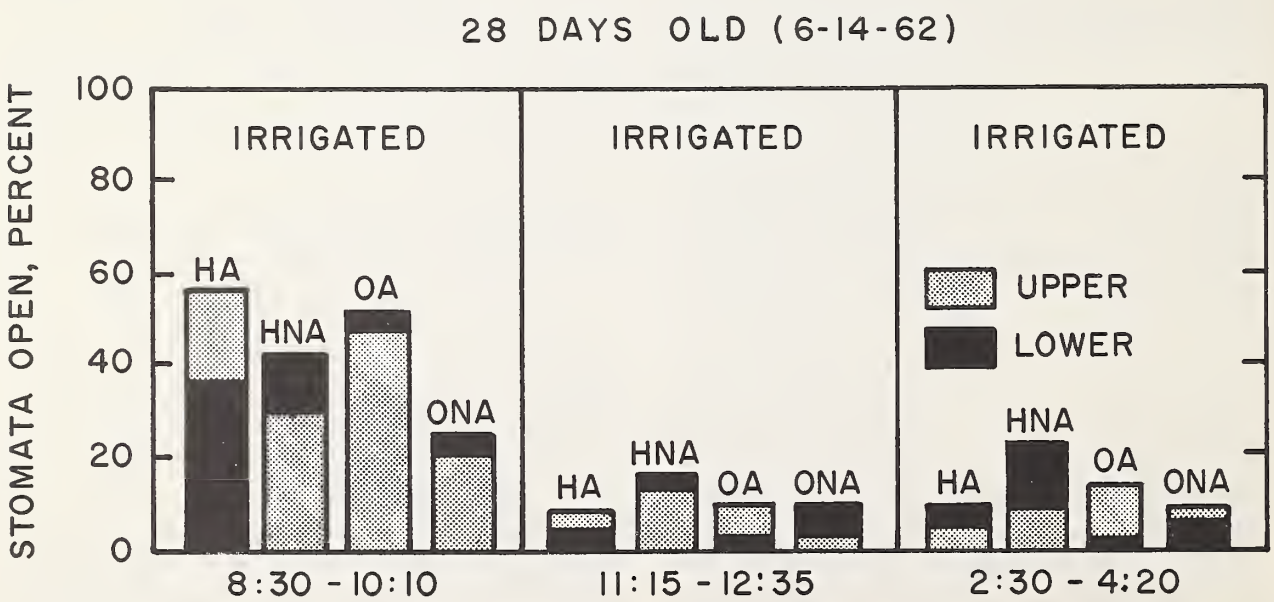


FIGURE 10.—Percentage of stomata open on the upper and lower epidermis of corn leaves at indicated times under indicated treatments. H=hybrid, O=open pollinated, A=Atrazine, NA=no Atrazine.

correlated with increasing soil moisture stress. Although the stomata on plants in plastic-covered plots ceased to open by the third week of July, several percent continued to open on the irrigated plants. Table 10 contains a summary of the stomatal condition on a single irrigated plant. Toward the latter part of the season shading of lower leaves may have had some influence in reducing the number of stomata visibly open. Radiant energy interception within the canopy changes considerably with the maturity of a corn field (*4*). Controlled environment studies (*23*) have shown that the percentage of corn stomata open decreases with decreasing light. Paralleling any increase in leaf surface in a growing crop is the probable decrease in ability of the plant to always meet the evaporative demand of its environment. Therefore, the trend to low-to-unperceptible stomatal activity as the season progressed may also have been due to a poor internal moisture relationship of the corn plant, bringing about or retaining hydroactive closure of its stomata.

Two differences stand out in these field studies when their stomatal activity is compared with

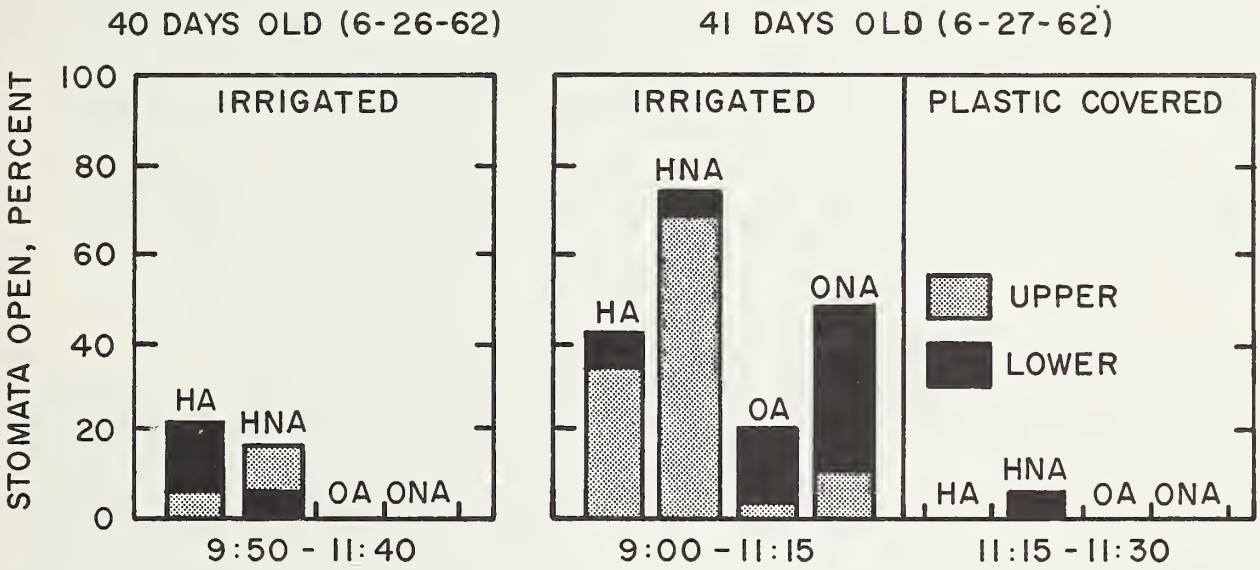


FIGURE 11.—Percentage of stomata open on the upper and lower epidermis of corn leaves at indicated times under indicated treatments. Considerably more stomata opened on the irrigated plots on 6–27 following a 2-inch rain on 6–26, whereas activity in the plastic-covered plot continued at a low level.

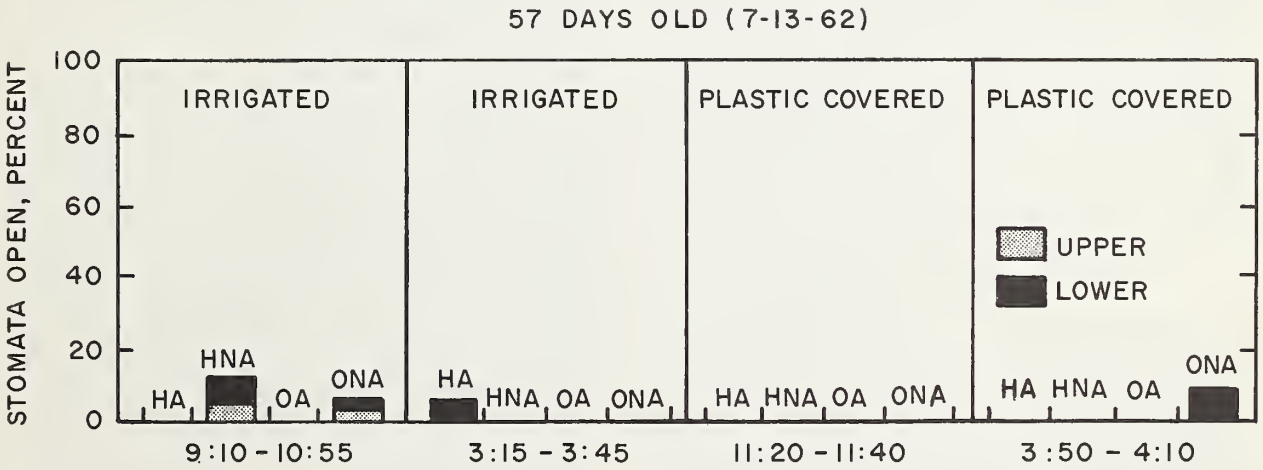


FIGURE 12.—Percentage of stomata open on the upper and lower epidermis of corn leaves at indicated times under indicated treatments. Visible stomatal activity by this age had subsided.

TABLE 10.—*Record of stomatal condition of corn leaves (7/19/62) at indicated time [1] on plant No. 7, a hybrid in an Atrazine-treated irrigated plot*

Time (a.m.)	Leaf No.	Upper epidermis [2]					Lower epidermis [2]					Total visibly open
		FO	O	O–C	C	Open	FO	O	O–C	C	Open	
		No.	*No.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*
11:10	19	0	0	0	10	0	0	0	0	10	0	0
11:13	18	0	0	0	10	0	0	1	0	9	10	10
11:15	17	0	0	0	10	0	0	1	0	9	10	10
11:17	16	0	1	0	9	10	0	0	0	10	0	3
11:19	15	0	0	0	10	0	0	0	0	10	0	5
11:21	14	0	0	0	10	0	0	0	0	10	0	0
11:23	13	0	0	0	10	0	0	0	0	10	0	8
11:25	12	0	0	0	10	0	0	1	0	9	10	3
11:28	11	0	1	0	9	10	0	1	0	9	10	8
11:30	10	0	0	0	10	0	0	0	0	10	0	0
11:32	9	0	0	0	10	0	0	0	0	10	0	3
11:34	8	0	0	0	10	0	0	0	0	10	0	0
11:35	7	0	0	0	10	0	0	0	0	10	0	3
11:37	6	0	0	0	10	0	0	0	0	10	0	0
11:39	5	0	0	0	10	0	0	0	0	10	0	0
11:41	4	0	0	0	10	0	0	0	0	10	0	3
11:43	3	0	0	0	10	0	0	0	0	10	0	0

Time (a.m.)	Leaf No.	Upper epidermis [2]					Lower epidermis [2]					Total visibly open
		FO	O	O–C	C	Open	FO	O	O–C	C	Open	
		No.	*No.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Pct.*
11:10	19	0	0	0	10	0	0	0	0	10	0	0
11:13	18	0	0	0	10	0	0	3	0	7	30	10
11:15	17	0	0	0	10	0	0	2	0	8	20	10
11:17	16	0	1	0	9	10	0	0	0	10	0	3
11:19	15	0	0	0	10	0	0	2	0	8	20	5
11:21	14	0	0	0	10	0	0	0	0	10	0	0
11:23	13	0	0	0	10	0	0	1	0	9	10	8
11:25	12	0	0	0	10	0	0	0	0	10	0	3
11:28	11	0	1	0	9	10	0	1	0	9	10	8
11:30	10	0	0	0	10	0	0	0	0	10	0	0
11:32	9	0	0	0	10	0	0	0	0	10	0	3
11:34	8	0	0	0	10	0	0	0	0	10	0	0
11:35	7	0	0	0	10	0	0	1	0	9	10	3
11:37	6	0	0	0	10	0	0	0	0	10	0	0
11:39	5	0	0	0	10	0	0	0	0	10	0	0
11:41	4	0	0	0	10	0	0	1	0	9	10	3
11:43	3	0	0	0	10	0	0	0	0	10	0	0

[1] The record indicates stomatal activity of corn leaves during the latter part of the season when the environmental conditions for the day of the determination were bright sunshine and high soil moisture availability, as 0.5 inch of rain had fallen the previous day.

[2] FO=full open; O=less than full open; O–C=indeterminably open or closed; C=closed.
The counts were made from a section of the leaf midway between the tip and the base. Each count was taken on opposite sides of the midvein.

controlled environment work. In many instances (figs. 10, 11) the percentage of stomata open on the upper surface of corn leaves was as great as that on the lower surface or greater; this was found very infrequently in controlled environment studies (*23*). Also, the percentage of stomata that were open on leaves during any one period of observation was higher than in any growth room studies. This discrepancy has led to the belief that serious limitations are frequently inherent in controlled environment studies (*22*).

Figure 13 shows that droughty conditions significantly delayed the initiation of silking in corn.

The lack of any Atrazine effect is consistent with the findings of Smith,[8] which became known to us after completion of this study. He conducted a 3-year field study on possible effects of Atrazine at Hancock and Madison, Wis.

Hexadecanol-Octadecanol

Hexadecanol and hexadecanol-octadecanol mixtures have been reported to increase water-use efficiency of field corn (*32*, *33*). The effectiveness of these long-chain alcohols has become a controversial issue because more recent research does not substantiate the original findings (*19*, *20*, *21*, *27*, *48*). The objective of research reported here was to further evaluate these compounds as transpiration suppressants for field crops, including an evaluation under soil moisture tension. In these experiments foliarly applied hexadecanol and soil-incorporated hexadecanol and a hexadecanol-octadecanol mixture were used under controlled environmental conditions.

Materials and Methods

Germinated test plants of *Phaseolus vulgaris* variety Red Kidney, *Zea mays* variety Dixie 82, and *Lycopersicon esculentum* variety Rutgers were grown under optimum temperature and light conditions in growth chambers. Day and night temperatures were 25° and 15° C. for beans; 30° and 15° for corn; 25° and 20° for tomatoes. Light was supplied 14 hours per day by Sylvania VHO cool white fluorescents (0.4 cal. $cm.^{-2}$ $min.^{-1}$). Individual plants were grown in asphalted 46-ounce juice cans containing 2 kg. of Cecil sandy clay loam soil. The standard fertilization rate was 120 pounds N, 100 pounds P, and 200 pounds K per acre; dolomitic limestone was added to obtain a soil pH of 6.

In the experiment with beans and corn the plants were subirrigated by placing the cans in 1 inch of water for 4 hours each night, after which

[8] Smith, Donald. Modification of Plant Transpiration Rate With Chemicals. 1963. (Unpublished Ph. D. dissertation; on file at University of Wisconsin, Madison, Wis.)

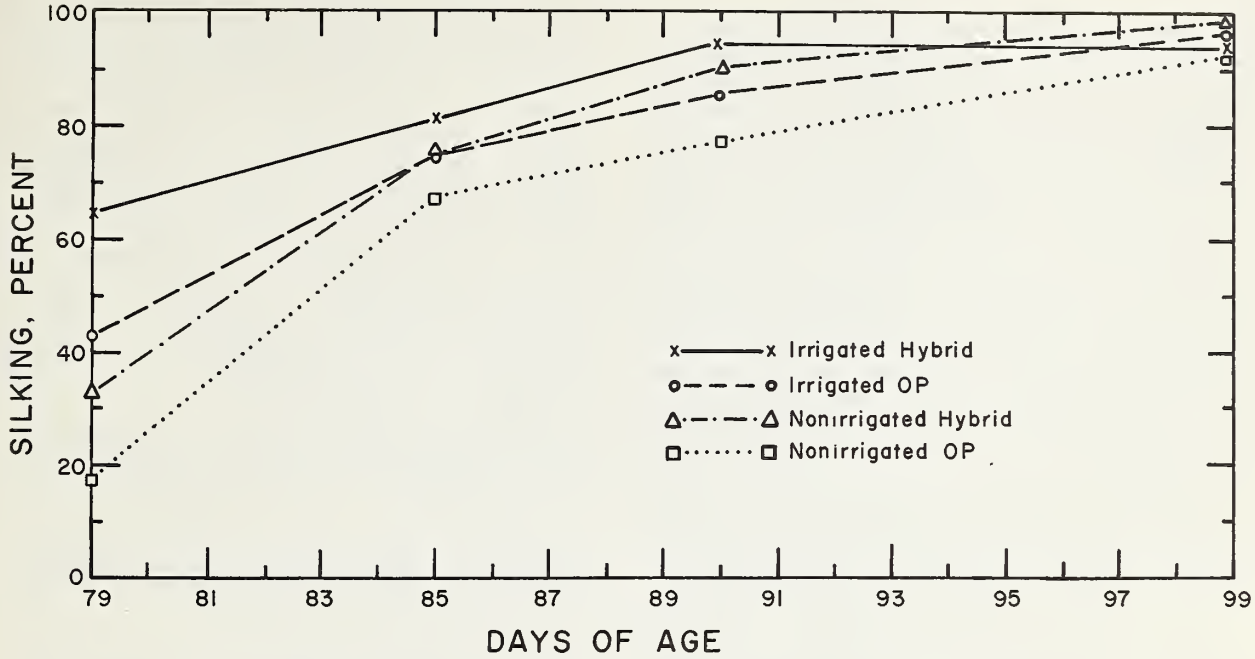


FIGURE 13.—Percentage of corn silking at indicated times as affected by treatment.

they were allowed to drain. Soil evaporation was minimized by placing a sheet of white polyethylene plastic around the base of each plant and over the top of the can. Beginning 3 weeks after planting, transpiration measurements were made daily by weighing the plants at 8 a.m. and 4:30 p.m.

Foliar Application

To test the effectiveness of hexadecanol as a foliar spray, 3-week-old bean and corn plants were sprayed with 90 percent ethanol (control) or 1.0, 0.1, or 0.01 percent hexadecanol in 90 percent ethanol until the spray ran off the leaves. All treatments were replicated six times.

Soil-Incorporated Hexadecanol

For soil incorporation studies, hexadecanol was thoroughly mixed at rates of 0, 0.18, and 0.37,[9] 0.74, 1.48, 2.96, 5.92, and 11.86 grams with 2 kg. air-dried soil. Pregerminated, uniform bean and corn plants were transplanted into the various mixtures. After the young seedlings were well established (approximately 2 weeks), transpiration was measured by weighing them daily. This procedure was continued for at least 1 week with corn, and up to 6 weeks with bean plants. Effects of the hexadecanol on emergence, growth, flowering, and fruiting were noted. Fresh and dry weights of the plants were determined at the end of the experiment.

[9] The approximate rate used by Roberts (*33*).

Soil-Incorporated Hexadecanol-Octadecanol

Effects of soil moisture tension on transpiration were studied by incorporating a hexadecanol-octadecanol mixture into the soil at the rates used in the hexadecanol soil-incorporation study. Two-week-old tomato plants were transplanted into the treated soil. After a week of conditioning, daily transpiration was recorded. The large population required two growth chambers. The plants in any one treatment were allowed to transpire until an average soil moisture tension of 10 atmospheres was reached; then they were rewatered to a soil moisture tension near 0.05 atmosphere. Cycles of wetting and drying were repeated until the plants were 9 weeks old.

Plant weight was estimated each week by washing the soil from the root system and weighing randomly selected plants. Soil moisture tension was calculated from soil moisture content by using desorption data (*30*). The desorption data were not corrected for possible changes caused by the incorporation of hexadecanol.

All data, when applicable, were treated by statistical analysis.

Results

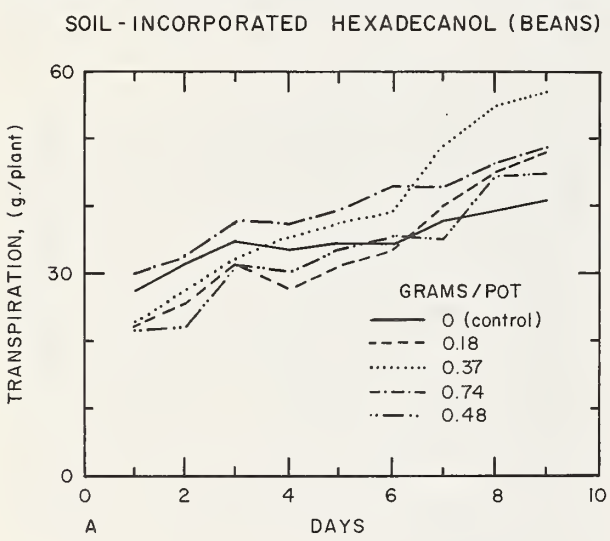
Foliar application.—Bean plants were killed by spraying with 1-percent solution of hexadecanol, but corn plants were not. Plants were not adversely affected by the ethyl alcohol spray control. No significant reduction in transpiration was found that was not associated with reduced growth

of bean plants. The only reduction in transpiration of corn plants, as a result of foliar spray, occurred on the day of treatment and with the highest concentration (1%). See the 1961 Annual Report (*26*) for quantitative data.

Soil-incorporated hexadecanol.—Transpiration of bean plants was significantly reduced by the high concentrations of soil-incorporated hexadecanol, as shown in figure 14B, but not by the lower concentrations (fig. 14A).

In the series of low concentrations, transpiration of corn was significantly reduced at the 1.48 rate (fig. 15A). At the higher rates (fig. 15B), only the 1.48 and the 11.85 rates significantly reduced transpiration. The compound also significantly reduced dry matter production and fruiting of bean plants and total growth of corn plants (table 11).

Soil-incorporated hexadecanol-octadecanol.—At soil moisture tensions not exceeding 10 atmospheres, a hexadecanol-octadecanol mixture reduced both transpiration and total growth of tomato plants. All transpiration coefficients in experiment I (table 12) are significantly lower than those of the comparable control. In experiment II (table 12) the transpiration coefficient of only the 2.96 rate is significantly lower. The 11.85 rate drastically reduced dry weight increase, and significantly increased transpiration per unit of dry matter produced.



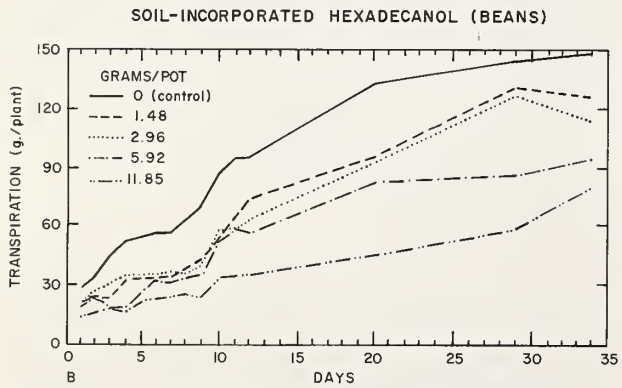


FIGURE 14.—Transpiration of bean plants as affected by soil incorporation of hexadecanol at indicated rates.

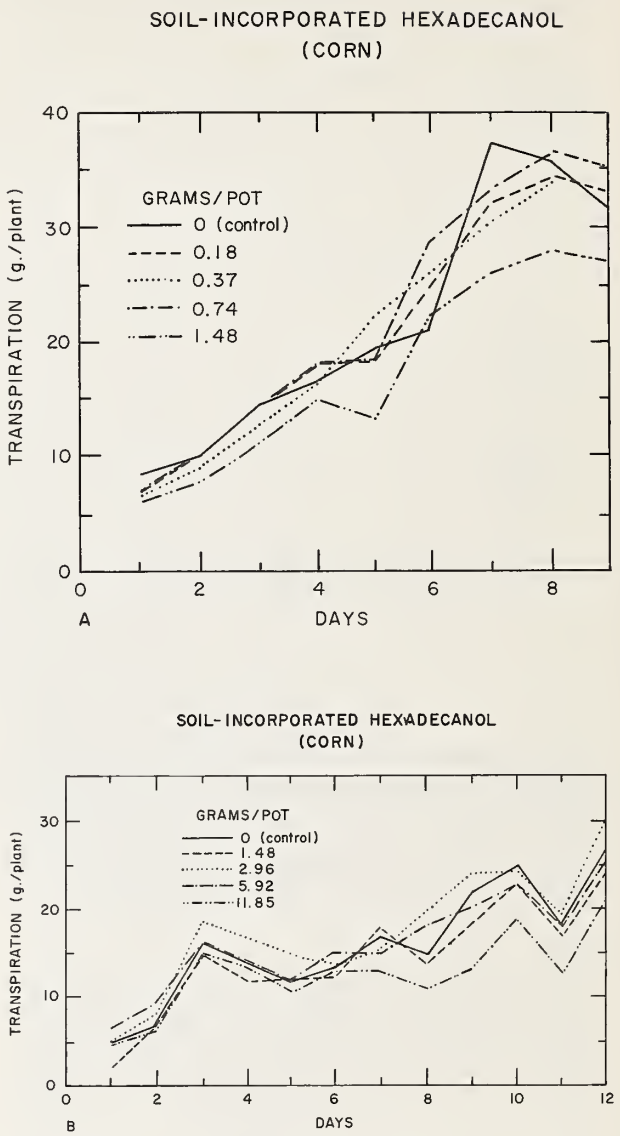


FIGURE 15.—Transpiration of corn plants as affected by soil incorporation of hexadecanol at indicated rates.

Discussion and Conclusion

The hypothesis proposed by Roberts (*33*) was that hexadecanol absorbed by plant roots might eventually clog stomata and thus reduce transpiration. This is plausible, since the internal

TABLE 11.—*Dry weight and fruiting of bean plants and dry weight of corn plants, as affected by concentration of hexadecanol* [1]

Treatment (Gram/2 kg. soil)	Bean plants, yield			Corn plants, yield of dry matter
	Dry matter	Fruit	Average fruit	
	Grams	*Grams*	*Number*	*Grams*
0 (control)	7. 4 a	28 a	18	2. 5 a
1.48	6. 4 b	23 b	14	2. 2 a
2.96	6. 1 b	20 bc	14	1. 8 ab
5.92	5. 9 b	18 c	10	1. 5 ab
11.85	2. 9 c	5 d	8	1. 4 b

[1] Values within a column followed by the same letter are not significantly different at the 5-percent level (Duncan's Multiple Range Test).

TABLE 12.—*Effect of a hexadecanol-octadecanol mixture on efficiency of soil water use by tomato plants as shown by the transpiration coefficient*

Experiment No. and treatment (grams/2 kg. soil)	Transpiration	Dry weight increase	Transpiration coefficient [1]
Experiment I:	*Grams*	*Grams*	
0	11, 983	37. 3	321 a
0.18	9, 377	35. 3	266 b
0.37	8, 551	29. 8	287 b
0.74	7, 093	27. 7	256 b
1.48	8, 576	32. 0	268 b
Experiment II:			
0	10, 399	40. 1	259 a
1.48	9, 281	38. 1	244 a
2.96	7, 151	35. 9	199 b
5.92	6, 754	27. 9	242 a
11.85	4, 717	12. 8	369 c

[1] Grams of water to produce 1 gram of dry weight. Values, within a given experiment, followed by the same letter are not significantly different at the 5-percent level (Duncan's Multiple Range Test).

transport of large molecules to leaf epidermis (*16*) as well as the clogging of stomata (*14*, p. 27) is already recorded. To show hexadecanol translocation, corn plants were autographed after exposure of the roots to C–14 labeled hexadecanol. Roberts' studies indicated that C–14 moved throughout the plant but did not include chemical identification of the compound producing the autograph or disallow for possible artifacts in autographing (*24*). Therefore, the original hypothesis is not proven. Hexadecanol and related compounds can reduce transpiration; however, as these studies showed, neither foliar nor soil applications economically increased the efficiency of water use whether stomata were clogged or not. Most of the applications of hexadecanol or hexadecanol-octadecanol tried were detrimental to both growth and development. Imposing soil moisture stress did not increase the efficiency of the compounds. Approaches mentioned (see page 15) other than that of clogging stomata look much more promising for moisture conservation by plantlife.

In the soil moisture tension study, water-use efficiency, as measured by the transpiration coefficient (table 12), increased at most rates of application but to the detriment of yield. This indicates that the transpiration coefficient alone is a poor measure of the net worth of a transpiration suppressant or a measure of water use efficiency; it may not take into consideration the economics of the treatment as reflected in yield reduction or decreases in the plant's resistance to unfavorable environment. It is presently difficult to visualize conditions where reductions in yield could be tolerated under field conditions to conserve water.

These findings are compatible with most reports to date (*19*, *20*, *21*, *27*, *48*). Thus, at present there is no basis for recommending the use of hexadecanol or hexadecanol-octadecanol mixtures as transpiration suppressants for field crops.

SUMMARY

Satisfactory growth chambers have been made from U.S. Army surplus walk-in refrigerators. Their characteristics and capabilities are discussed.

Growth and fruiting by tomato and bean plants grown in a controlled environment under VHO Gro-Lux lamps were inferior to those of plants grown under cool white VHO fluorescent lamps.

Studies have shown that for several crop species, low CO_2 in the atmosphere tends to cause stomata to open, and high atmospheric CO_2 tends to close stomata; however, the ability of low CO_2 to maintain stomatal aperture is of secondary importance to the opposite effect of high soil moisture tension. The critical CO_2 concentration for stomatal action differs for the several species and varieties tested.

With adequate soil moisture, transpiration can be reduced by establishing CO_2 at a level sufficiently high to maintain stomata in a closed position; however, leaf temperature rises several degrees when stomata remain closed for extended periods while the plant is exposed to radiation.

The amount of light received daily by several species of plants had a very definite effect on their stomatal activity. Considerably more cyclosis in the guard cell and open stomata were observed day and night when daylight consisted of 360 cal. $cm.^{-2}$ day^{-1} than when daylight consisted of 36 cal. $cm.^{-2}$ day^{-1}.

A field study of the effects of Atrazine on water use and stomatal activity by a hybrid and an open-pollinated corn grown at two moisture levels revealed that the hybrid corn used moisture more efficiently than the open-pollinated corn. Atrazine had no apparent effect on stomatal activity, but high soil moisture tension drastically reduced stomatal activity. Atrazine did not improve water use efficiency by corn.

Studies with foliar applications of hexadecanol and soil applications of hexadecanol and octadecanol revealed that these compounds can reduce transpiration; however, most rates of application were detrimental to both growth and development of plants. No economically feasible rate of hexadecanol-octadecanol can be recommended for field crops.

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